EXENDIN-3

His Ser Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
1 5 10 15
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
20 25 30
Ser Gly Ala Pro Pro Pro Ser$_2$NH
35

Fig. 1

EXENDIN-4

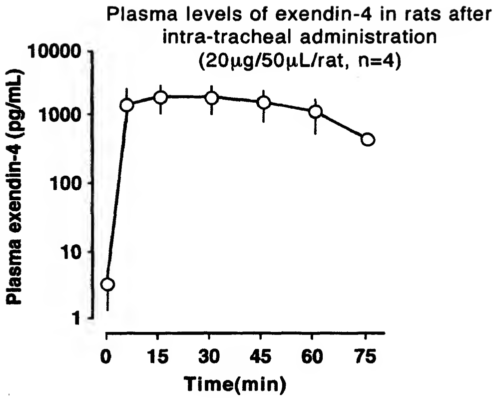
His	Gly	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Met	Glu	Glu
				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Gly	Pro	Ser
			20					25					30		
Ser	Gly	Ala	Pro	Pro	Pro	Ser-NH_2									
		35													

Fig. 2

GLP-1 (GLP-1[7-36] NH_2)

His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
5 10 15
Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg-NH_2
20 25 30

Fig. 3



Male rats (350-400g) fasted overnight were cannulated in the trachea and femoral artery under anesthesia.
Blood was drawn from the arterial line before and after (5, 15, 30, 45, 60 and 75 min) 20μg of exendin-4 dissolved in 50μL saline was administered into the trachea of each rat.
Plasma exendin-4 levels were determined with an immunoradiometric assay.

Fig. 4

Plasma exendin-4 concentration after intratracheal instillation (1ug/20ul/mouse)

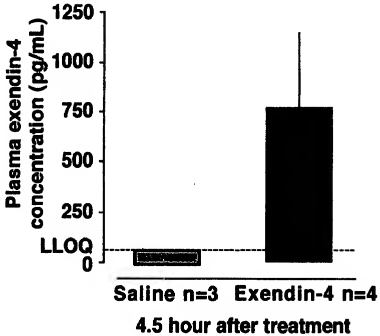


Fig. 5A

Effect of intra-trachea administration of exendin-4 (1ug/20ul/mouse) on plasma glucose in db/db mice

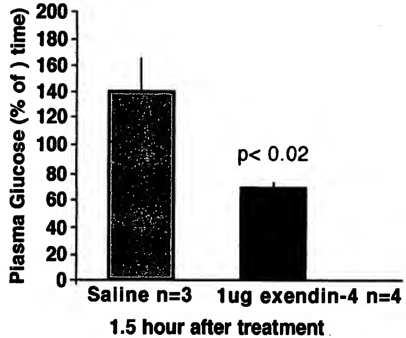


Fig. 5B

Male db/db mice (approx 50g) were fasted for 2h, and the trachea was intubated under anesthesia. The animals were bled (75 µL, orbital sinus) before and after 20 µL of saline or 1µg exendin-4 dissolved in saline was administered into the trachea of each animal.

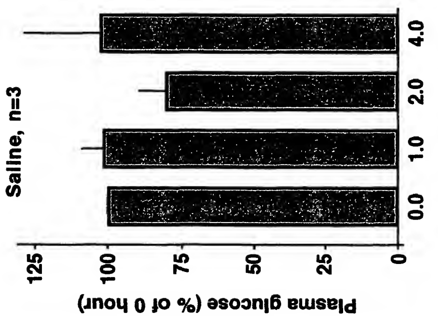

Saline, n=3
Plasma glucose (% of 0 hour)
125
100
75
50
25
0
0.0
1.0
2.0
4.0

Fig. 6B

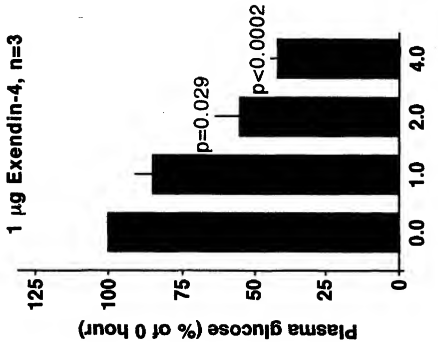

1 µg Exendin-4, n=3
Plasma glucose (% of 0 hour)
125
100
75
50
25
0
0.0
1.0
2.0
4.0
p=0.029
p<0.0002

Fig. 6B

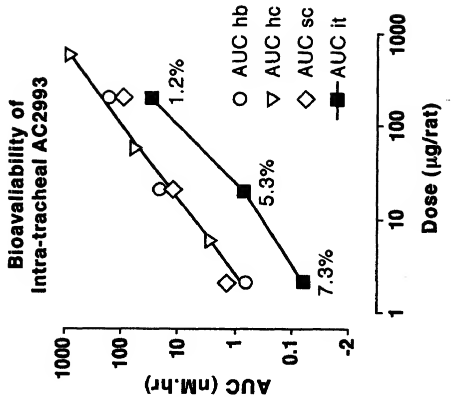

Bioavailability of
Intra-tracheal AC2993
AUC (nM.hr)
1000
100
10
1
0.1
-2
1
10
100
1000
Dose (µg/rat)
7.3%
5.3%
1.2%
AUC hb
AUC hc
AUC sc
AUC it

Fig. 7B

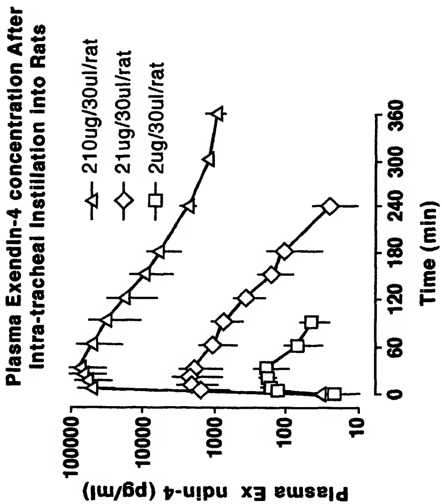
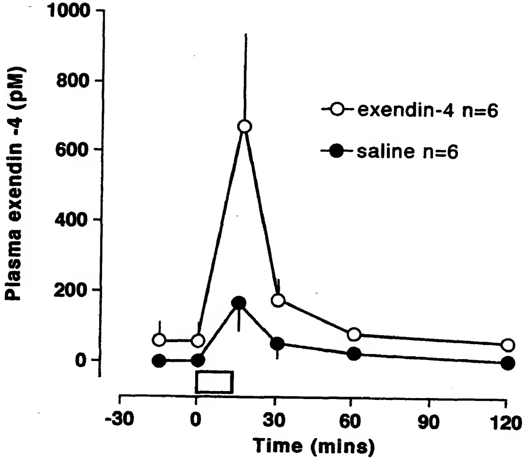

Plasma Exendin-4 concentration After
Intra-tracheal Instillation Into Rats
210ug/30ul/rat
21ug/30ul/rat
2ug/30ul/rat
Plasma Ex ndin-4 (pg/ml)
100000
10000
1000
100
10
0
60
120
180
240
300
360
Time (min)

Fig. 7A

Piasma x ndin-4 conc ntrations in rat xposed to a rosoliz d x ndin-4(8ng/ml) for 10 minutes



Male rats (approximately 350g each) fasted overnight were placed in a 2 litre chamber and exposed to aerosolized exendin-4 for 10 minutes.
Exendin-4 was nebulized at a rate of 0.2mg/min at a flow rate of 5L/min.
The concentration of aerosolized exendin-4 was estimated from samples of chamber atmosphere drawn during the course of the experiment.

Fig. 8

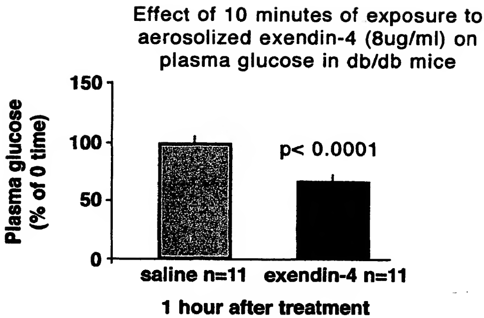

Effect of 10 minutes of exposure to aerosolized exendin-4 (8ug/ml) on plasma glucose in db/db mice
Plasma glucose (% of 0 time)
150
100
50
0
p< 0.0001
saline n=11
exendin-4 n=11
1 hour after treatment

Fig. 9A

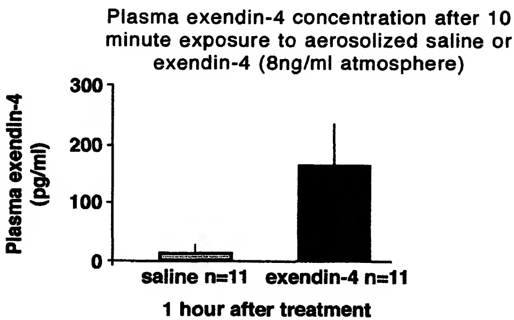
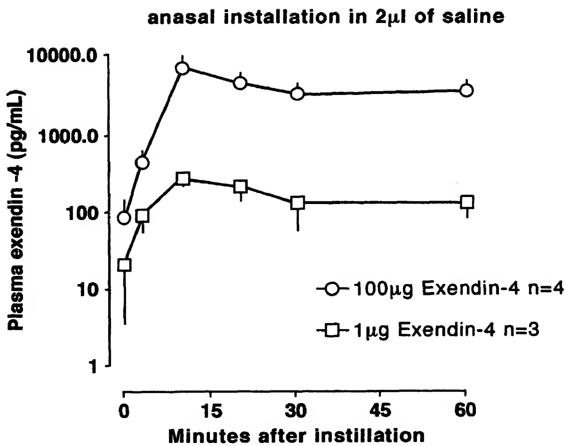
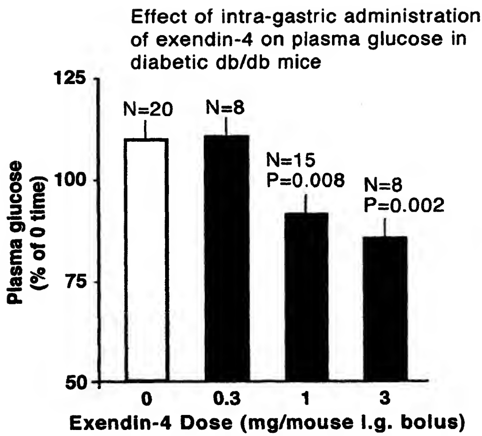

Plasma exendin-4 concentration after 10 minute exposure to aerosolized saline or exendin-4 (8ng/ml atmosphere)
Plasma exendin-4 (pg/ml)
300
200
100
0
saline n=11
exendin-4 n=11
1 hour after treatment

Fig. 9B



Harlan Sprague Dawley rats 311-365g, nonfasted, were dosed with 0, 1, 100μg of exendin-4 in 2μl of saline by application in to the nostrils.
Blood samples from anesthetized (Hurricane) tail tip were collected at 0, 3,10, 20, 30 and 60 min after dosing for exendin-4 plasma level measured by IRMA.

Fig. 10



Male db/db mice (approx 50g) were fasted for 2h and bled (40µl, orbital sinus) before and 1h after 200 µl of saline or exendin-4 dissolved in saline was administered i.g. into each animal.

Sublingual

Sublingual application of exendin-4 (100µg/5 µL/animal) to diabetic db/db mice led to a 15% decrease in plasma glucose concentration one hour after treatment. A 30% increase was observed for the control group receiving saline. The mean exendin-4 plasma level at 60min was 4520±1846 pg/mL (see Figure 8).

Fig. 11

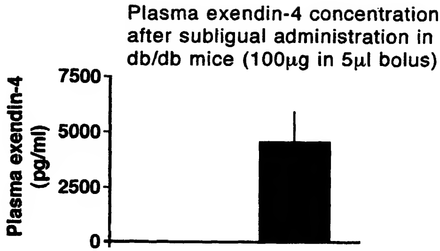


Fig. 12A

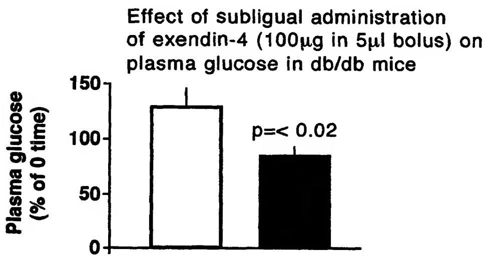
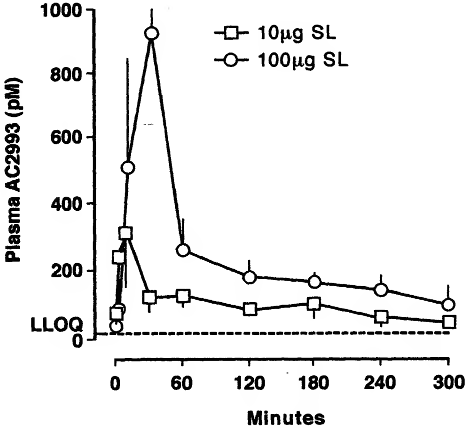


Fig. 12B

Plasma Concentration after Sublingual Administration of AC2993 in Rats



Dose-was given in 3μL saline under the tongue in HSD rats (~300g) briefly anesthetized with metophane.

Fig. 12C

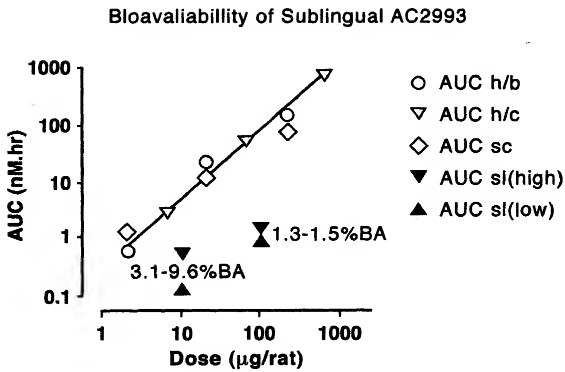

Bloavaliabillity of Sublingual AC2993
AUC (nM.hr)
1000
100
10
1
0.1
1
10
100
1000
Dose (µg/rat)
AUC h/b
AUC h/c
AUC sc
AUC sl(high)
AUC sl(low)
1.3-1.5%BA
3.1-9.6%BA

Fig. 12D

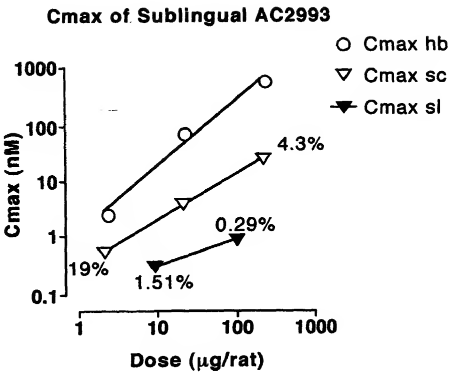

Cmax of Sublingual AC2993
Cmax hb
Cmax sc
Cmax sl
1000
100
10
1
0.1
Cmax (nM)
4.3%
0.29%
19%
1.51%
1
10
100
1000
Dose (µg/rat)

Fig. 12E

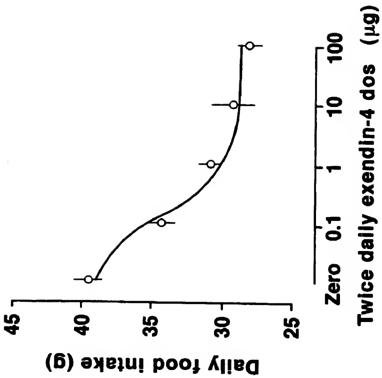


Fig. 13A2

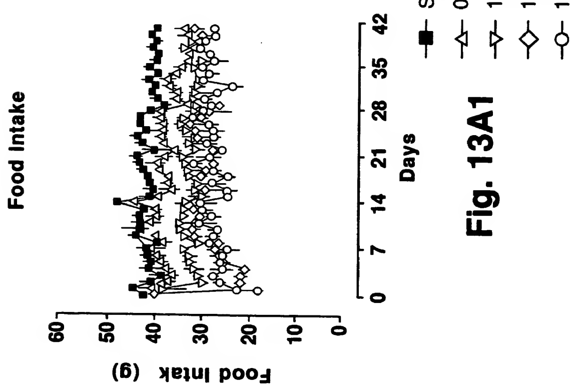


Fig. 13A1

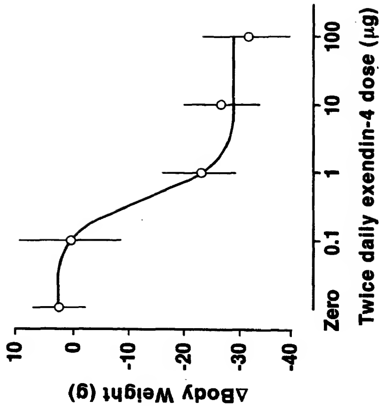


Fig. 13B2

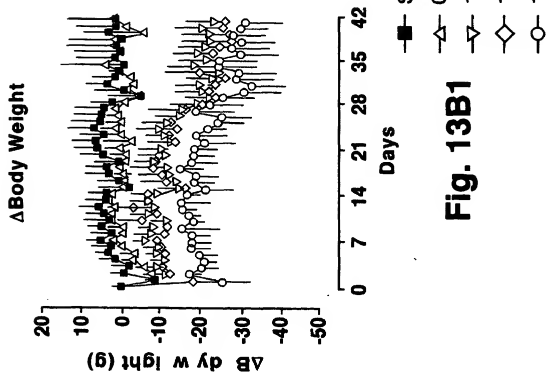


Fig. 13B1

09/889330

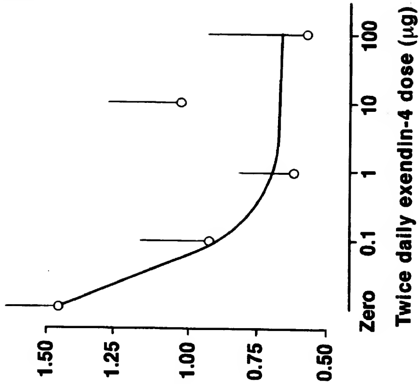


Fig. 13C2

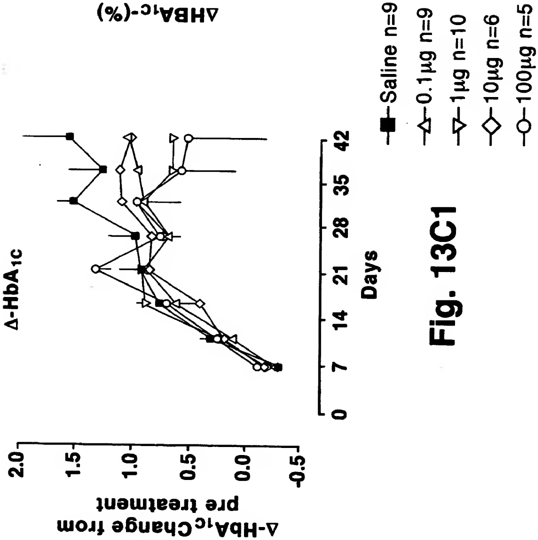


Fig. 13C1

SUBSTITUTE SHEET (RULE 26)

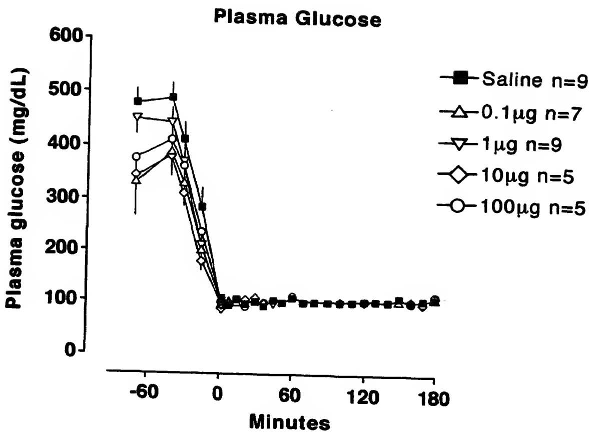

Plasma Glucose
600
500
400
300
200
100
0
Plasma glucose (mg/dL)
Saline n=9
0.1µg n=7
1µg n=9
10µg n=5
100µg n=5
-60
0
60
120
180
Minutes

Fig. 14A

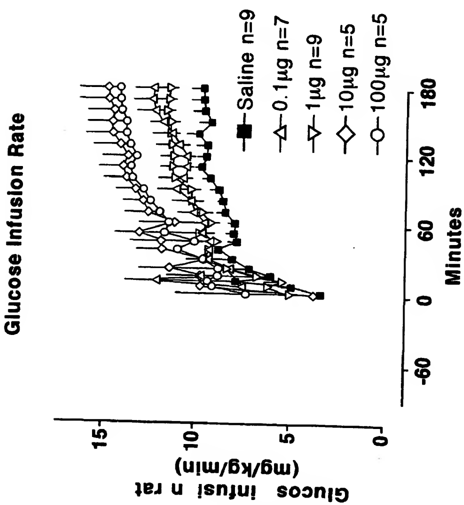


Fig. 14B1

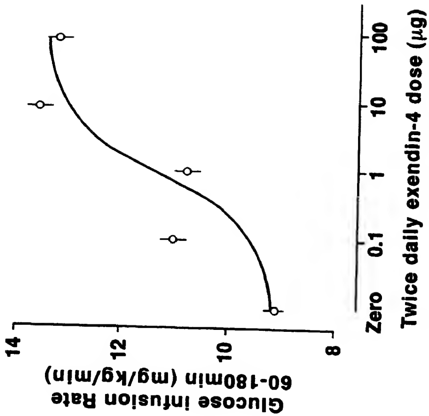


Fig. 14B2

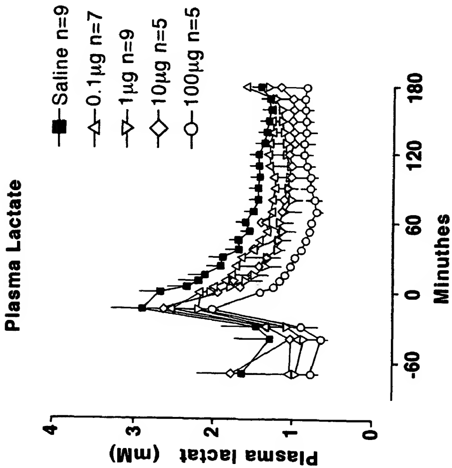


Fig. 14C1

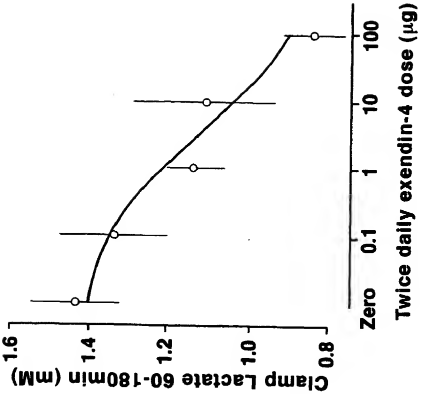


Fig. 14C2

1 5 10 15 20
Xaa_1 Xaa_2 Xaa_3 Gly Thr Xaa_4 Xaa_5 Xaa_6 Xaa_7 Xaa_8 Ser Lys Gln Xaa_9 Glu Glu Glu Ala Val Arg Leu

25 30 35
Xaa_{10} Xaa_{11} Xaa_{12} Xaa_{13} Leu Lys Asn Gly Gly Xaa_{14} Ser Ser Gly Ala Xaa_{15} Xaa_{16} Xaa_{17} Xaa_{18}-Z

[SEQ. ID. NO.]	Xaa_1	Xaa_2	Xaa_3	Xaa_4	Xaa_5	Xaa_6	Xaa_7	Xaa_8	Xaa_9	Xaa_{10}	Xaa_{11}	Xaa_{12}	Xaa_{13}	Xaa_{14}	Xaa_{15}	Xaa_{16}	Xaa_{17}	Xaa_{18}
9	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	Ile	Glu	Phe	Pro	Pro	Pro	Pro	Ser
10	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Ser
11	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Phe	Pro	Pro	Pro	Pro	Ser
12	Tyr	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Ser
13	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Tyr
14	His	Gly	Asp	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Ser
15	His	Gly	Glu	naph	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Ser
16	His	Gly	Glu	Phe	Ser	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Ser
17	His	Gly	Glu	Phe	Ser	Thr	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Ser
18	His	Gly	Glu	Phe	Thr	Thr	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Ser
19	His	Gly	Glu	Phe	Thr	Ser	Glu	Leu	Met	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Ser
20	His	Gly	Glu	Phe	Thr	Ser	Asp	pGly	Met	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Ser
21	His	Gly	Glu	Phe	Thr	Ser	Asp	pGly	Leu	Phe	Ile	Glu	Phe	Pro	Pro	Pro	Pro	Ser
22	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	pGly	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Ser
23	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	pGly	Phe	Ile	Glu	Phe	Pro	Pro	Pro	Pro	Ser
24	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	naph	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Ser
25	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Val	Glu	Trp	Pro	Pro	Pro	Pro	Ser

Fig. 15A

[SEQ. ID. NO.]	Xaa1	Xaa2	Xaa3	Xaa4	Xaa5	Xaa6	Xaa7	Xaa8	Xaa9	Xaa10	Xaa11	Xaa12	Xaa13	Xaa14	Xaa15	Xaa16	Xaa17	Xaa18
26	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	Val	Glu	Phe	Pro	Pro	Pro	Pro	Ser
27	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	tBuG	Glu	Trp	Pro	Pro	Pro	Pro	Ser
28	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	tBuG	Glu	Phe	Pro	Pro	Pro	Pro	Ser
29	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Asp	Trp	Pro	Pro	Pro	Pro	Ser
30	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Phe	Pro	Pro	Pro	Pro	Ser
31	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	tPro	tPro	tPro	tPro	Ser
32	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	tPro	tPro	tPro	Ser
33	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	hPro	hPro	hPro	hPro	Ser
34	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	hPro	hPro	hPro	Ser
35	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	Ile	Glu	Phe	tPro	tPro	tPro	tPro	Ser
36	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	Ile	Glu	Phe	hPro	hPro	hPro	hPro	Ser
37	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	MeAla	MeAla	MeAla	MeAla	Ser
38	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	MeAla	MeAla	MeAla	Ser
39	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	Ile	Glu	Phe	MeAla	MeAla	MeAla	MeAla	Ser

Fig. 15B

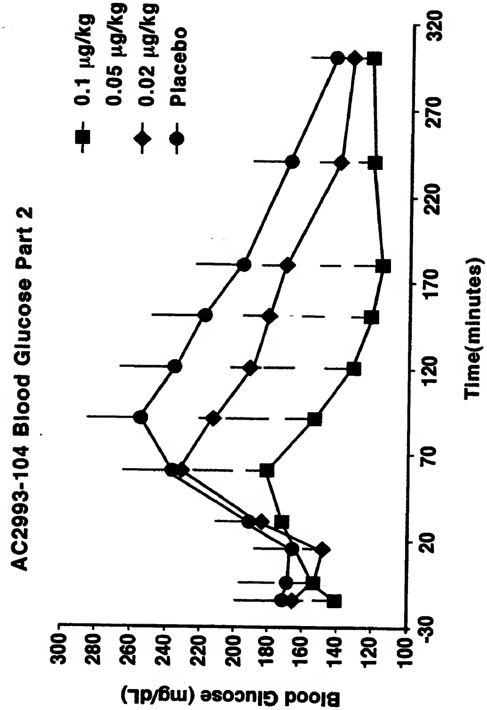

AC2993-104 Blood Glucose Part 2
0.1 µg/kg
0.05 µg/kg
0.02 µg/kg
Placebo
Blood Glucose (mg/dL)
300
280
260
240
220
200
180
160
140
120
100
-30
20
70
120
170
220
270
320
Time(minutes)

Fig. 16

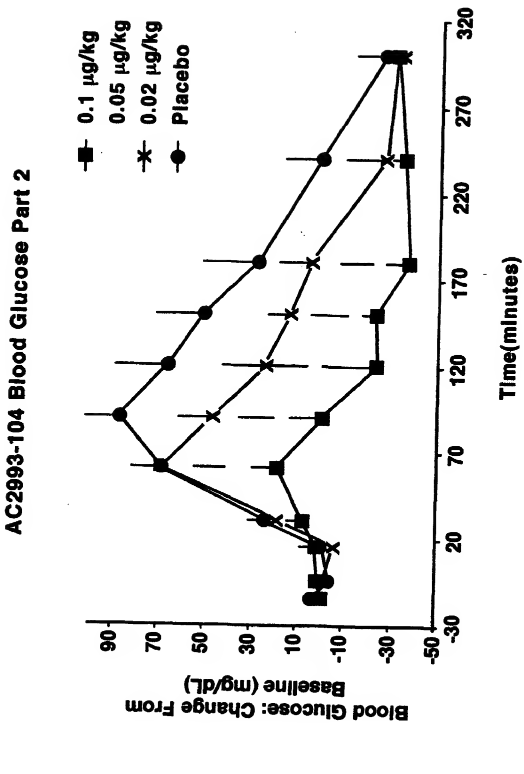

AC2993-104 Blood Glucose Part 2
0.1 μg/kg
0.05 μg/kg
0.02 μg/kg
Placebo
Blood Glucose: Change From Baseline (mg/dL)
90
70
50
30
10
-10
-30
-50
-30
20
70
120
170
220
270
320
Time(minutes)

Fig. 17